SOME ASPECTS OF EXPERIMENTAL CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY*

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The mechanisms by which carbon tetrachloride (CCl_4) and other poisons produce their deleterious effects upon the liver have been recently reexamined. The impetus for the current interest in this problem arose from the studies of Christie and Judah[1] and Dianzani.[2] These investigators presented evidence showing that CCl_4 was capable of directly affecting mitochondrial oxidative enzymes of the liver parenchymal cell and postulated that the primary defect was probably the result of the release of coenzyme from the mitochondria. That these changes can be brought about *in vitro* and *in vivo* has been confirmed by many groups.[3, 4, 5, 6]

From a consideration of the vigorous treatment necessary to produce these changes *in vitro* and from evidence which appeared in the literature relating to the sympathetic nature of fat depot innervation, we began a series of experiments which led to the hypothesis set forth in 1959 that CCl_4 effects on the liver are largely indirect.[7, 8] The hypothesis that the sympathetic nervous system played a major role in CCl_4-induced hepatotoxicity stemmed from the following observations:

1. It was shown that the effects of CCl_4 on the liver, both the centrilobular necrosis and the lipid accumulation could be markedly reduced by prior administration of adrenergic blocking agents such as dibenzyline, ergotamine, and phentolamine.[7] The effect on necrosis was more pronounced than the protection against lipid accumulation.

2. Prior high spinal transection in the rat could block completely both of these changes produced by CCl_4.[7, 9, 10] Liver damage was assessed both by changes in mitochondrial function (such as the loss in diphosphopyridine nucleotide-linked oxidative enzyme function or the ATP-ase transformation[5]), and by histological examination. Lipid accumulation was determined by measuring both total lipids[9] and triglycerides.

3. It was also demonstrated that the catecholamine content of the adrenal medulla of the CCl_4-treated rat was reduced. Furthermore, prior cord section which protected the liver also prevented this loss of epinephrine and norepinephrine from the adrenal.[7,12]

Another early observation supported the concept that the centrilobular

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changes and the lipid accumulation were separable phenomena. Recknagel *et al.*[11] had reported that the lipid rise was an early effect, while the mitochondrial changes were a later manifestation of liver damage. Studies in adrenalectomized animals tended to confirm this concept.[7, 9] Adrenalectomy only minimally modified the mitochondrial changes and necrosis seen after CCl_4, yet no lipid accumulation was evident either by chemical measurement or histological examination.

The studies to be presented here are offered as further evidence that the action of carbon tetrachloride upon the liver *in vivo* is primarily an indirect one and also supports our hypothesis that the sympathetic nervous system plays a key role in the toxicity.

Results

A. Ganglionic blockade. Since we were able to effectively protect against the liver damage by prior spinal transection, ganglionic blockade was selected as a means of blocking nervous transmission to determine whether such a pharmacologic agent might protect as effectively. A relatively long-acting ganglion-blocking agent was selected, trimethidium bismethosulfate (Ostensin, Wyeth). A long-acting compound was deemed necessary since CCl_4-treated animals were usually sacrificed 20 hours after a single administration of the poison and such a drug would avoid multiple injections in order to maintain good blockade. The protective effects of ganglionic blockade on liver mitochondria from CCl_4-treated rats are illustrated in TABLE 1. Also for comparative purposes is shown a typical experiment where the same parameters were measured in a cord-sectioned rat receiving CCl_4.

This data would indicate that as far as CCl_4-induced damage in mitochondrial activity is concerned, prior administration of this ganglionic blocking agent was apparently almost as effective as prior spinal transection. Ganglionic blockade was also equally effective in preventing the ATP-ase transformation. However, this agent did not markedly reduce the lipid accumulation usually observed with CCl_4.[13] FIGURE 3 shows a section of rat liver from an animal receiving trimethidinium and then challenged with the standard dose of CCl_4 (2.5 ml./kg., orally in peanut oil). FIGURE 1 shows a section of a liver from a rat receiving only CCl_4. It can be seen that ganglionic blockade affords excellent protection, but not as effective as that seen with prior cord section shown in FIGURE 2, the latter tissue being indistinguishable from that obtained from the liver of an untreated control. While no centrilobular changes are seen with this ganglionic blocking agent, there is still marked triglyceride accumulation (FIGURE 3). FIGURE 4 shows (for comparative purposes) the liver

TABLE 1
EFFECTS OF GANGLIONIC BLOCKADE AND CORD-SECTION ON CCl_4 INDUCED MITOCHONDRIAL DAMAGE

	Treatment	Glutamate		Glutamate + DPN		Succinate	
		O_2*	P:O†	O_2	P:O	O_2	P:O
Exp. 1	Gang. block. control‡	6.2	2.4	6.4	2.3	5.3	2.1
	CCl_4	0	0	3.8	2.3	5.3	1.3
	Gang. block. + CCl_4	5.6	2.6	6.9	2.1	6.2	2.0
Exp. 2	Cord section control	9.3	3.1	9.8	2.7	9.0	2.4
	CCl_4	0	0	2.9	2.1	7.1	1.0
	Cord section + CCl_4	8.7	2.9	8.7	2.8	8.1	2.0

*Microatoms oxygen consumed in 7 minutes.
†Ratio of micromoles of inorganic phosphate taken up to microatoms of oxygen consumed.
‡Trimethidinum bismethosulfate, 50 mgm./kgm. S.Q., in two doses, 2 hours prior to and 10 hours after CCl_4.

from an animal receiving CCl_4 after adrenalectomy. Here, it is clear that necrotic changes have occurred, yet there is no apparent accumulation of fat. This has been confirmed by chemical analysis. It should be pointed out that Rees[14] has found that prior adrenalectomy fails to protect against this increase in lipids.

B. Anti-release agents. The effects of a number of so-called sympathetic anti-release agents were also investigated. These compounds, while

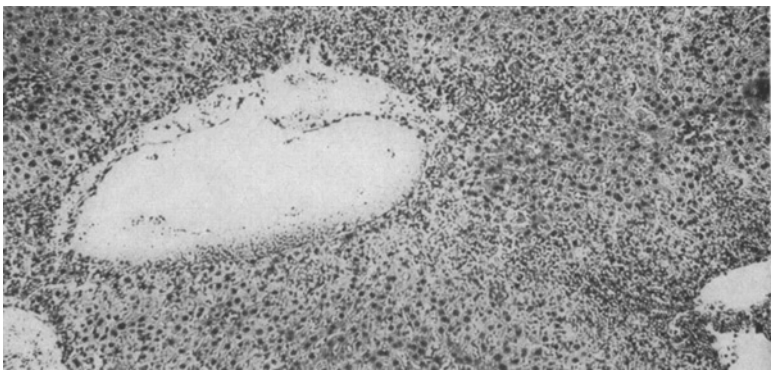

FIGURE 1. Liver from rat receiving 2.5 ml./kg. of CCl_4 orally 20 hours prior to sacrifice. H & E stain. × 100.

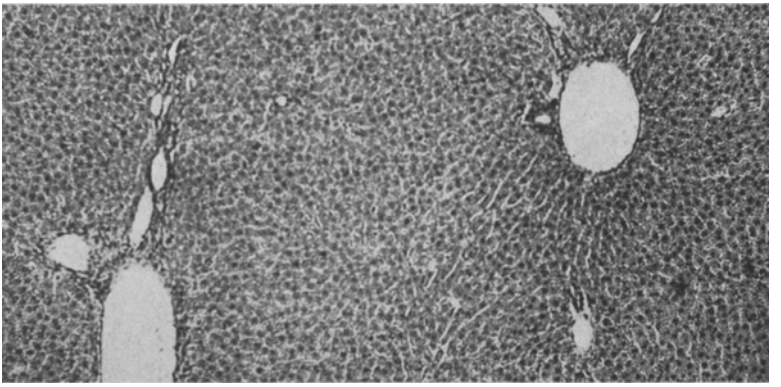

FIGURE 2. Liver from cord-sectioned rat receiving the standard dose of CCl_4 20 hours prior to sacrifice. H & E stain. × 100.

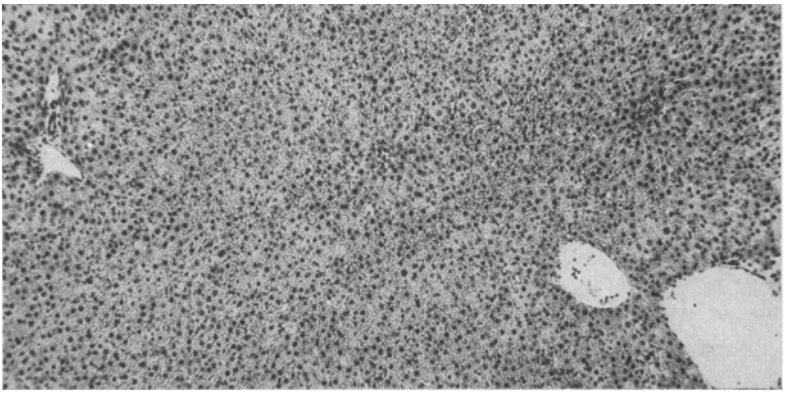

FIGURE 3. Liver from a rat after ganglionic blockade and CCl_4. H & E stain. × 100.

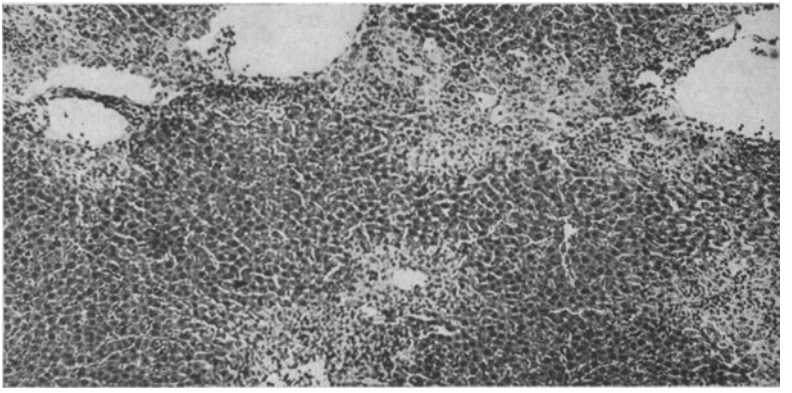

FIGURE 4. Liver from a CCl_4-treated rat after prior adrenalectomy. H & E stain. × 100.

they may possess other pharmacologic properties as well, are all capable of preventing norepinephrine secretion from sympathetic post-ganglionic fibers. The drugs studied included β-TM-10, (2-2,6-dimethylphenoxy propyl)-trimethyl-ammonium chloride,* guanethidine (Ismelin),† and bretylium (Darenthin). TABLE 2 shows the effects of β-TM-10 on mitochondrial oxidative phosphorylation of livers from rats receiving CCl_4 after the anti-release drug. This is a typical experiment and illustrates

TABLE 2
EFFECT OF PRIOR ADMINISTRATION OF AN ANTI-RELEASE AGENT ON CCl_4-INDUCED MITOCHONDRIAL DAMAGE

Treatment	Glutamate		Glutamate + DPN		Succinate	
	O_2*	P:O†	O_2	P:O	O_2	P:O
β-TM-10 Control	6.6	2.7	6.0	2.6	3.7	2.0
CCl_4	0	0	2.4	3.2	4.3	1.2
β-TM-10 + CCl_4	7.3	2.9	6.3	2.7	3.8	2.2

*Microatoms of oxygen consumed in 7 minutes.
†Ratio of micromoles of inorganic phosphate taken up to microatoms of oxygen consumed.

that this drug too is capable of protecting against the mitochondrial damage seen with CCl_4.[13] However, prior pretreatment was essential and large doses of the drug were necessary. Similar results could be obtained with both guanethidine and bretylium, although both of these were somewhat less effective than β-TM-10.

Histologic sections of livers of animals pretreated with β-TM-10 and guanethidine and challenged with the standard dose of CCl_4 are shown in FIGURES 5 and 6 respectively. β-TM-10 affords excellent protection against centrilobular change, however there is still quite a marked accumulation of lipid (FIGURE 5). Guanethidine (FIGURE 6) was less effective, but still gave significant protection. The histologic changes seemed to correlate quite well with the biochemical events for this group of compounds.

C. Catecholamines. While decreased catecholamine levels in adrenals of CCl_4-treated rats had been previously reported by our group,[12] circulating levels of catecholamines were not determined at that time. Recent

*SKF 6890A, kindly supplied by E. J. Fellows, Smith, Kline, and French Labs. Philadelphia, Pa.
†Kindly supplied by F. F. Yonkman, Ciba Labs., Summit, N. J.

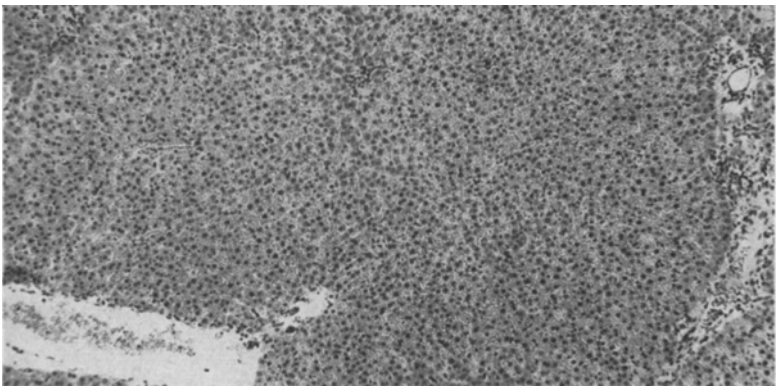

FIGURE 5. Liver from a rat 20 hours after receiving CCl_4 and after pretreatment with β-TM-10. H & E stain. × 100.

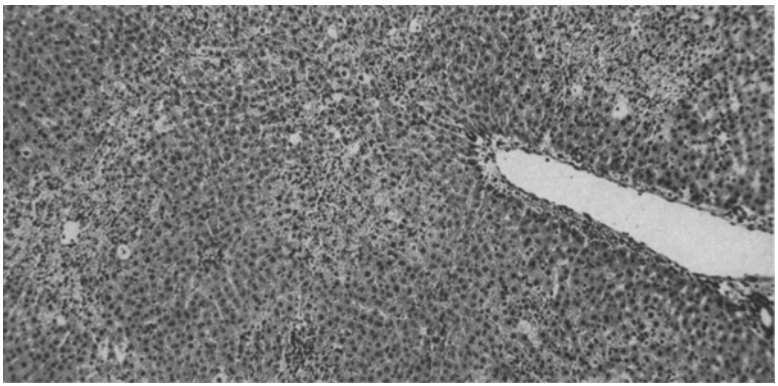

FIGURE 6. Liver from a rat 20 hours after receiving CCl_4 and after pretreatment with guanethidine. H & E stain. × 100.

studies have shown that CCl_4 is capable of elevating both plasma and urine epinephrine and norepinephrine levels.[15, 16] The elevated urinary free catecholamine levels were shown to be the result of increased secretion rather than decreased metabolism or tissue depletion. It could also be shown that agents that were effective in reducing the necrosis usually seen with CCl_4 could also reduce the elevated norepinephrine excretion of CCl_4-treated animals. Some of these data are shown in TABLE 3.

It may be observed from TABLE 3 that although these agents are not all effective in blocking the CCl_4-induced epinephrine increase, they do markedly depress the rise in norepinephrine excretion that is usually seen with CCl_4. This increase in excretion of free amine could be detected as

TABLE 3
URINARY EXCRETION OF FREE CATECHOLAMINES*

Treatment	Epinephrine†	Norepinephrine†
Control	1.0	2.4
CCl_4	10.4	8.3
CCl_4 + Cord section	0.2	0.4
CCl_4 + β-TM-10	13.8	4.3
CCl_4 + Guanethidine	8.3	3.5
CCl_4 + Trimethidium	0.6	1.4

*Assayed by the procedure of Bertler *et al.*[17]
†Micrograms free base/kg./24 hours.

early as two hours after CCl_4 treatment and was continuous until the animal was sacrificed.

D. Corticosterone levels after CCl_4 administration. Since CCl_4 does elevate plasma catecholamines and increase the excretion of free epinephrine and norepinephrine, it seemed likely that it might also effect a steroid release. Plasma corticosterone levels were determined after CCl_4 administration. As shown in TABLE 4, CCl_4 does evoke a rise in corticosterone levels in rat plasma. While the effect is marked (about 3–4 fold), it is not long-lasting. Furthermore, cord-sectioned animals also exhibit this elevation in steroid levels. Although this confirms the fact that CCl_4

TABLE 4
PLASMA CORTICOSTERONE FOLLOWING CCl_4 ADMINISTRATION

Treatment	Number of animals	Plasma corticosterone* μgm./100 ml.
none	6	20 ± 8
CCl_4 (1 hr.)	5	67 ± 11
CCl_4 (2 hr.)	5	70 ± 7
CCl_4 (5 hr.)	2	64, 59
CCl_4 (10 hr.)	3	42 ± 10
Peanut oil (1 hr.)	5	39 ± 4
Peanut oil (2 hr.)	5	29 ± 7
CCl_4 + Adx	2	3, 1
Cord section	2	43, 44
Cord section + CCl_4 (5 hr.)	4	47 ± 10

*Mean ± s.d.

is a stressing agent, it is unlikely that the elevation in steroid observed plays a prominent role in CCl_4 hepatotoxicity.

E. Lipid accumulation. The effect of CCl_4 upon the increase of liver triglyceride has been widely studied. A recent report by Recknagel[18] has suggested that liver triglycerides rise because of the failure of a "triglyceride-secreting mechanism" of the hepatic parenchymal cell. Other possibilities may be a decrease in synthesis of protein carrier[19, 20] or an increase in the mobilization of free fatty acid from peripheral depots.[8, 9, 21] That epinephrine and norepinephrine are capable of mobilizing depot fat is now well-established.[22-25] It is certainly conceivable that several of these factors may be important when the ultimate mechanism is established. However, since corticoids do influence lipid accumulation (see studies on adrenalectomized animals above), and from the evidence that has accumulated that corticoids play a permissive role in catecholamine action, it would be surprising if epinephrine and/or norepinephrine were not in some way responsible for the triglyceride accumulation after CCl_4 administration.

Summary

The experiments with the ganglionic blocking compound and the anti-release agents would tend to support the hypothesis that the sympathetic nervous system plays a key role in CCl_4-induced hepatotoxicity in the rat. Studies on plasma levels and the urinary excretion of free norepinephrine are offered as further evidence that hepatic damage is at least in part a consequence of sympathetic stimulation. While corticosterone is also elevated by CCl_4, its role in liver necrosis appears to be a minor one.

Acknowledgments

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